

AMENDMENT TO THE CLAIMS

1. (Currently amended) An engineered nucleic acid molecule comprising:
 - (i) a first stem-forming portion;
 - (ii) a second stem-forming portion, wherein the two stem-forming portions are complementary or substantially complementary, and
 - (iii) a non-stem-forming portion ~~that forms a loop~~ connecting the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion,

wherein the engineered nucleic acid molecule ~~forms a stem-loop structure that~~ represses translation when inserted ~~positioned~~ upstream of an open reading frame.
2. (Currently amended) The nucleic acid molecule of claim 1, wherein the nucleic acid molecule forms a stem-loop structure positioned upstream of the ORF.
3. (Original) The nucleic acid molecule of claim 1, wherein the first and second stem-forming portions are substantially complementary.
4. (Original) The nucleic acid molecule of claim 1, wherein at least a portion of the first stem-forming portion is complementary or substantially complementary to a ribosome binding site (RBS).
5. (Original) The nucleic acid molecule of claim 1, wherein at least a portion of the first stem-forming portion is complementary or substantially complementary to a Kozak consensus sequence.
6. (Original) The nucleic acid molecule of claim 1, wherein the sequence of the second stem-forming portion comprises an RBS.
7. (Original) The nucleic acid molecule of claim 1, wherein the sequence of the non-stem-forming portion comprises YUNR.
- 8 – 14. (Canceled).
15. (Currently amended) The nucleic acid molecule of claim 1, wherein the stem exhibits

between 60 and 100% ~~75 and 95%~~ complementarity.

16. (Canceled).

17. The nucleic acid molecule of claim 1, wherein the stem includes at least one area of non-complementarity.

18. (Canceled).

19. (Original) The nucleic acid molecule of claim 1, wherein the stem includes at least two dispersed areas of non-complementarity.

20-23. (Canceled).

24. (Original) The nucleic acid molecule of claim 1, wherein the nucleic acid molecule represses translation in the absence of a ligand.

25. (Currently amended) The nucleic acid molecule of claim 1, wherein the nucleic acid molecule is composed of RNA or DNA.

26-27. (Canceled).

28. (Original) The nucleic acid molecule of claim 1, wherein the nucleic acid molecule comprises a nucleotide analog.

29. (Original) The nucleic acid molecule of claim 1, wherein the first stem-forming portion comprises a sequence complementary or substantially complementary to a sequence in the 5' portion of an ORF.

30 – 35 (Canceled).

36. (Original) The nucleic acid molecule of claim 1, further comprising a third stem-forming portion that is complementary or substantially complementary to the second stem-forming portion, wherein the first and third stem-forming portions form alternate stem-loop structures with the second stem-forming portion.

37 – 40 (Canceled).

41. (Currently amended) The nucleic acid molecule of claim 1, wherein the nucleic acid

molecule has the sequence of crR10 or crR12.

42. (Currently amended) A DNA construct that comprises a template for transcription of the nucleic acid molecule of claim 41, ~~wherein the nucleic acid molecule is composed of RNA.~~

43 – 44 (Canceled).

45. (Currently amended) The nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a variant of crR10 or crR12 and differs from crR10 or crR12 such that the variant permits formation of a stable secondary structure that represses translation by 12 or less nucleotides and includes at least 1 area 3-dispersed areas of non-complementarity.

46. (Currently amended) A nucleic acid ~~DNA~~ construct that comprises a template for transcription of the nucleic acid molecule of claim 45.

47 – 48 (Canceled).

49. (Currently amended) A nucleic acid ~~DNA~~ construct that comprises a template for transcription of the nucleic acid molecule of claim 1, ~~wherein the nucleic acid molecule is composed of RNA.~~

50. (Original) A cell comprising the DNA construct of claim 49.

51. (Canceled).

52. (Currently amended) A plasmid comprising the nucleic acid ~~DNA~~ construct of claim 49.

53. (Currently amended) The nucleic acid construct ~~plasmid~~ of claim ~~49~~ 52, wherein the nucleic acid construct ~~plasmid~~ comprises a promoter operably linked to the template for transcription of the nucleic acid molecule.

54. (Currently amended) The nucleic acid construct ~~plasmid~~ of claim 53, wherein the promoter is inducible or synthetic.

55 – 56. (Canceled).

57. (Currently amended) The nucleic acid construct ~~plasmid~~ of claim 53, wherein the promoter is

responsive to an environmental or developmental signal.

58. (Currently amended) The nucleic acid construct ~~plasmid~~ of claim 53, wherein the promoter functions in prokaryotic cells.

59. (Currently amended) The nucleic acid construct ~~plasmid~~ of claim 53, wherein the promoter functions in eukaryotic cells.

60 – 61 (Canceled).

62. (Currently amended) An engineered nucleic acid molecule comprising:

(i) a first stem-forming portion;

(ii) a second stem-forming portion; and

(iii) a non-stem-forming portion, wherein the non-stem-forming portion connects the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion ~~to form a loop~~, and wherein a portion of the nucleic acid molecule is complementary or substantially complementary, to a portion of the ~~a cognate~~ nucleic acid molecule of claim 1.

63 – 67 (Canceled).

68. (Currently amended) The nucleic acid molecule of claim 62, wherein the two stem-forming portions exhibit between 60 and 100% ~~75 and 95%~~ complementarity.

69 - 71. (Canceled)

72. (Original) The nucleic acid molecule of claim 62, wherein the stem includes at least two dispersed areas of non-complementarity.

73. (Canceled)

74. (Original) The nucleic acid molecule of claim 62, wherein the stem includes at least three dispersed areas of non-complementarity.

75- 81. (Canceled).

82. (Original) The nucleic acid molecule of claim 62, wherein the nucleic acid molecule activates

translation of an mRNA whose translation is repressed by a cognate cis-repressive nucleic acid molecule.

83 – 85 (Canceled).

86. (Currently amended) The nucleic acid molecule of claim 62, wherein the nucleic acid molecule has the sequence of taR10 or taR12.

87 - 89. (Canceled).

90. (Currently amended) The nucleic acid molecule of claim 62, wherein the nucleic acid molecule is a variant of taR10 or taR12 and differs from taR10 or taR12 such that the variant permits formation of a stable secondary structure by 12 or less nucleotides and includes at least 1 area ~~3 dispersed areas~~ of non-complementarity.

91 -93 (Canceled).

94. (Currently amended) A nucleic acid ~~DNA construct~~ that comprises a template for transcription of the nucleic acid molecule of claim 62, ~~wherein the nucleic acid molecule is composed of RNA.~~

95. (Original) The nucleic acid ~~DNA construct~~ of claim 94, further comprising a template for transcription of the nucleic acid molecule of claim 1, ~~wherein the nucleic acid molecule is composed of RNA.~~

96 – 115 (Canceled).

116. (Currently amended) A system for control of gene expression comprising:

(i) a first nucleic acid molecule comprising a cis-repressive sequence element upstream of an open reading frame (ORF), or including part of the open reading frame, wherein the first nucleic acid molecule forms a stem-loop structure that represses translation of the ORF; and

(ii) a second nucleic acid molecule comprising first and second stem-forming portions and a non-stem-forming portion, wherein the non-stem-forming portion connects the 3' end of the first stem-forming portion and the 5' end of the second stem-forming

portion to form a loop, and wherein a portion of the second nucleic acid molecule is complementary or substantially complementary to a portion of the first nucleic acid molecule and interacts with the first nucleic acid molecule to derepress translation of the ORF.

117 – 176 (Canceled).

177. (Original) A kit for allowing a user to regulate expression of a gene of choice comprising:

(a) a first plasmid comprising

(i) a template for transcription of a cis-repressive RNA element; and

(ii) a promoter located upstream of the template for transcription of the cis-repressive RNA element;

(b) a second plasmid comprising

(i) a template for transcription of a cognate trans-activating RNA element;

and

(ii) a promoter located upstream of the template for transcription of the trans-activating RNA element; and

(c) one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

178. (Original) A kit for allowing a user to regulate expression of a gene of choice comprising:

a plasmid comprising a template for transcription of a cis-repressive RNA element and a promoter located upstream of the template for transcription of the cis-repressive RNA element and further comprising a template for transcription of a cognate trans-activating RNA element and a promoter located upstream of the template for transcription of the cognate trans-activating RNA element; and

one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

179. (Original) A kit for allowing a user to regulate expression of a gene of choice comprising:

(a) a first plasmid comprising

(i) a template for transcription of a cis-repressive RNA element; and

(ii) a promoter located upstream of the template for transcription of the cis-repressive RNA element;

(b) a second plasmid comprising

(i) a template for transcription of a cognate trans-activating RNA element;

and

(ii) a promoter located upstream of the template for transcription of the trans-activating RNA element;

(c) a third plasmid comprising a template for transcription of a cis-repressive RNA element and a promoter located upstream of the template for transcription of the cis-repressive RNA element and further comprising a template for transcription of a cognate trans-activating RNA element and a promoter located upstream of the template for transcription of the cognate trans-activating RNA element; and

(d) one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

180. (Original) A kit comprising:

one or more oligonucleotides comprising a crRNA sequence, one or more oligonucleotides comprising a taRNA sequence, or one or more oligonucleotides comprising a crRNA sequence and one or more oligonucleotides comprising a taRNA sequence, wherein the kit further comprises one or more items selected from the group consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

181. (Currently amended) A method of regulating translation of an open reading frame comprising steps of:

introducing an engineered template for transcription of an mRNA into a cell and allowing mRNA transcription to occur resulting in a transcribed mRNA, wherein the template is engineered so that the transcribed mRNA comprises first and second nucleic acid elements that form a stem-loop structure that represses translation of the mRNA; and providing an engineered nucleic acid molecule that interacts with the mRNA so as to derepress translation of the mRNA to the cell.

182. (Currently amended) The method of claim 181, wherein the engineered template comprises:

- (i) a first stem-forming portion;
- (ii) a second stem-forming portion, wherein the two stem-forming portions are complementary or substantially complementary; ~~and~~
- (iii) a non-stem-forming portion ~~that forms a loop~~ connecting the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion; and
- (iv) an open reading frame (ORF),

wherein the engineered nucleic acid molecule forms a stem-loop structure that represses translation of the ~~when positioned upstream of an~~ ORF.

183. (Original) The method of claim 182, wherein the first and second stem-forming portions are substantially complementary.

184. (Original) The method of claim 182, wherein at least a portion of the first stem-forming portion is complementary or substantially complementary to a ribosome binding site .
185. (Original) The method of claim 182, wherein at least a portion of the first stem-forming portion is complementary or substantially complementary to a Kozak consensus sequence.
186. (Original) The method of claim 182, wherein the sequence of the second stem-forming portion comprises an RBS.
- 187 – 209. (Canceled).
210. (Currently amended) The method of claim 181, wherein the step of providing comprises: inducing transcription of the engineered nucleic acid molecule ~~in the cell~~.
211. (Currently amended) The method of claim 181, wherein the step of providing comprises: delivering the engineered nucleic acid molecule to a cell exogenously.
- 212 - 241 (Canceled).